PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agcnt's file reference EJH/EK	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. **PCT/AU 96/00085**	International filing date *(day/month/year)* 20 February 1996	(Earliest) Priority Date *(day/month/year)* 20 February 1995

Applicant
(1) AMRAD OPERATIONS PTY LTD
(2) HARRISON, Leonard et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **4** sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ **Certain claims were found unsearchable** (See Box I)

2. ☐ **Unity of invention is lacking** (See Box II)

3. ☐ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   ☐ filed with the international application

   ☐ furnished by the applicant separately from the international application,

      ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed

   ☐ transcribed by this Authority

4. With regard to the **title,**

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established by this Authority to read as follows:

Immunoreactive and immunotherapeutic molecules which interact in subjects with Insulin-Dependent Diabetes Mellitus (IDDM)

5. With regard to the **abstract,**

   ☒ the text is approved as submitted by the applicant

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No.

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure

   ☐ because this figure better characterises the invention

   ☒ None of the figures

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00085

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/62, 14/725, 14/47, 7/06, 7/08; G01N 33/68, 33/564; C12Q 1/25; A61K 38/43, 38/10, 38/28 //C12Q 1/02; C12R 1:91

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6]: C07K, A61K, C12Q, G01N. All through electronic databases. Chemical Abstracts

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Medline Through electronic database

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, Medline Keywords: SS1: GAD or GLUTAMIC (W) ACID (W) DECARBOXYLASE# SS2: PROINSULIN# or PRO(W)INSULIN# SS3: IDDM or DIABETES(W)MELLITUS SS4: 1 or 2 SS5: 3 and 4 STN SEARCH: FYTPKTRRE; YTPKTRREAE; TPKTRREAED; FWYIPPSLRT; WYIPPSLRTL; YIPPSLRTLE; IPPSLRTLED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 95/07992 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 25 March 1995, IPC[6] C12N, C07K, G01N, A61K See entire document especially page 26 line 7 - page 28 line 9, Examples, Table 11, claims, Figure 3	1-3,6-12,15-23, 26-32,35,36
X,Y	WO 92/05446 (REGENTS OF THE UNIVERSITY OF CALIFORNIA) 2 April 1992, IPC[5] G01N, C12N, C07H, A61K See entire document especially Example 3 and Figures 2, 3 and 4	1,5-10,14-17 19,20,25-28, 30,34-36

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 1996	1ST MAY 1996.

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	**ROBYN PORTER** Telephone No.: (06) 283 2318

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/14485 (AMRAD CORPORATION LIMITED) 3 September 1992, IPC[5] A61K, C07K, C12N, G01N See entire document	1,6-8,15,16,18, 26,27,30,35,36
X	WO 94/12529 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 9 June 1994, IPC[5] C07K, G01N See entire document, especially page 35 line 18 to page 38 line 6, Example 7, claims 21, 37, 42, 45, 47 and 50 and Figure 1	1,8-10, 19-21,30,36
X	K. Daw & A.C. Powers: "Two Distinct Glutamic Acid Decarboxylase Auto-Antibody Specificities in IDDM Target Different Epitopes", Diabetes, vol 44, no 2, 216-220, February 1995 See entire document including Figure 3	8,14-16,19, 25-27
Y	L. Mauch et al: "Characterization of a linear epitope within the human pancreatic 64-kDa glutamic acid decarboxylase and its autoimmune recognition by sera from insulin-dependent diabetes mellitus patients". Eur. J. Biochem., 212, 2, 597-603 (1993)	1,6-8,15, 16,19,26,27
P,X	WO 95/07464 (UNIVERSITY OF WASHINGTON) 16 March 1995, IPC[6] G01N, C07K See abstract, Examples 3 and 4, Sequence ID Nos 1 and 2 and claims	8-10,19-21
X	WO 92/20811 (ZYMOGENETICS & THE BOARD OF REGENTS OF THE UNIVERSITY OF WASHINGTON) 26 November 1992, IPC[5] C12P, C12N, C12Q, C07K, C07H, A01N See page 15 line 27 - page 16 line 36, page 20 line 29 - page 21 line 29, Example 3, claims 16, 21 and 25 and Figure 2	16-21, 26-30,35,36

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 96/00085

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9507992	AU	79201/94				
WO	9205446	AU	88771/91	AU	21706/95	CA	2040555
		EP	502188	US	5475086	AU	15163/92
		CA	2070004	EP	519469	JP	7070182
WO	9214485	AU	12748/92	CA	2104225	EP	572478
WO	9412529	EP	701569				
WO	9507464	AU	76441/94				
WO	9220811	AU	19976/92	CA	2103159	EP	585356
		IL	101872	NZ	242747		

END OF ANNEX

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PN5172 EJH/EK

Box No. I TITLE OF INVENTION

IMMUNOREACTIVE AND IMMUNOTHERAPEUTIC MOLECULES

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

AMRAD OPERATIONS PTY. LTD.
17-27 Cotham Road
KEW 3101
VICTORIA
AUSTRALIA

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HARRISON, Leonard
27 Park Street
ST KILDA 3182
VICTORIA
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HUGHES, E John L
SLATTERY, John M
CORBETT, Terence G

DAVIES COLLISON CAVE
1 Little Collins Street
Melbourne 3000
Victoria Australia

Telephone No. +61 3 9542 2777

Facsimile No. +61 3 9254 2770

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HONEYMAN, Margot
27 Park Street
ST KILDA 3182
VICTORIA
AUSTRALIA

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

RUDY, George
10/3 Osborne Avenue
GLEN IRIS 3146
VICTORIA
AUSTRALIA

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: UNITED STATES OF AMERICA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LEW, Andrew
13 Warner Street
ESSENDON 3040
VICTORIA
AUSTRALIA

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** **AZ** Azerbaijan, **BY** Belarus, **KZ** Kazakstan, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] EP **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania
- [X] AM Armenia .....
- [X] AT Austria .....
- [X] AU Australia .....
- [X] AZ Azerbaijan
- [X] BB Barbados
- [X] BG Bulgaria .....
- [X] BR Brazil .....
- [X] BY Belarus
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China .....
- [X] CZ Czech Republic .....
- [X] DE Germany .....
- [X] DK Denmark .....
- [X] EE Estonia .....
- [X] ES Spain .....
- [X] FI Finland .....
- [X] GB United Kingdom
- [X] GE Georgia .....
- [X] HU Hungary .....
- [X] IS Iceland
- [X] JP Japan .....
- [X] KE Kenya .....
- [X] KG Kyrgyzstan .....
- [X] KP Democratic People's Republic of Korea .....
- [X] KR Republic of Korea .....
- [X] KZ Kazakstan .....
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho .....
- [X] LT Lithuania
- [X] LU Luxembourg .....
- [X] LV Latvia
- [X] MD Republic of Moldova .....
- [X] MG Madagascar .....
- [X] MK The former Yugoslav Republic of Macedonia .....
- [X] MN Mongolia
- [X] MW Malawi .....
- [X] MX Mexico .....
- [X] NO Norway
- [X] NZ New Zealand .....
- [X] PL Poland .....
- [X] PT Portugal .....
- [X] RO Romania
- [X] RU Russian Federation .....
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia .....
- [X] SK Slovakia .....
- [X] TJ Tajikistan .....
- [X] TM Turkmenistan .....
- [X] TR Turkey .....
- [X] TT Trinidad and Tobago .....
- [X] UA Ukraine .....
- [X] UG Uganda .....
- [X] US United States of America .....
- [X] UZ Uzbekistan .....
- [X] VN Viet Nam .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [ ] .....
- [ ] .....
- [ ] .....
- [ ] .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) AUSTRALIA	20 February 1995 20-02-1995	PN1239	
item (2) AUSTRALIA	4 September 1995 04-09-1995	PN5172	
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

[X] The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1) PN1239 & (2) PN5172

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used).* ISA /_____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	: 4	sheets
2. description	: 22	sheets
3. claims	: 7	sheets
4. abstract	: 1	sheets
5. drawings	: 5	sheets
Total	: 39	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

AMRAD Operations Pty Ltd
By:

Name: J. M. SMITH
Position: COMPANY SECRETARY

HARRISON, Leonard

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority specified by the applicant: ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:

in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient:

in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:

in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III.

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant:

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor:

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:

in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV:

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":

in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application:

(vi) if there are more than three earlier applications whose priority is claimed:

in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:

in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.

CONTINUATION OF BOX NO. IX

In an Attorney

HONEYMAN, Margot

RUDY, George

Andrew M. Lew

LEW, Andrew

663272

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/62, 14/725, 14/47, 7/06, 7/08, G01N 33/68, 33/564, C12Q 1/25, A61K 38/43, 38/10, 38/28 // C12Q 1/02, C12R 1:91

A1

(11) International Publication Number: WO 96/26218

(43) International Publicati n Date: 29 August 1996 (29.08.96)

(21) International Application Number: PCT/AU96/00085

(22) International Filing Date: 20 February 1996 (20.02.96)

(30) Priority Data:

PN 1239	20 February 1995 (20.02.95)	AU
PN 5172	4 September 1995 (04.09.95)	AU

(71) Applicant *(for all designated States except US)*: AMRAD OPERATIONS PTY. LTD. [AU/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HARRISON, Leonard [AU/AU]; (AU). HONEYMAN, Margo [AU/AU]; 27 Park Street, St Kilda, VIC 3182 (AU). RUDY, George [US/AU]; 10/3 Osborne Avenue, Glen Iris, VIC 3146 (AU). LEW, Andrew [AU/AU]; 13 Warner Street, Essendon, VIC 3040 (AU).

(74) Agents: HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IMMUNOREACTIVE AND IMMUNOTHERAPEUTIC MOLECULES WHICH INTERACT IN SUBJECTS WITH INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

(57) Abstract

The present invention relates generally to molecules such as peptides, polypeptides and proteins which interact immunologically with antibodies or T-cells in subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM). These molecules are preferentially immunoreactive to T-cells in subjects having pre-clinical or clinical IDDM and are useful in the development of diagnostic, therapeutic and prophylactic agents for IDDM.

IMMUNOREACTIVE AND IMMUNOTHERAPEUTIC MOLECULES WHICH INTERACT IN SUBJECTS WITH INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

The present invention relates generally to molecules such as peptides, polypeptides and proteins which interact immunologically with antibodies or T-cells in subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM). These molecules are preferentially immunoreactive to T-cells in subjects having pre-clinical or clinical IDDM and are useful in the development of diagnostic, therapeutic and prophylactic agents for IDDM.

Amino acid sequences are referred to herein by sequence identity numbers (SEQ ID NOs) which are defined at the end of the specification.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers, but not to the exclusion of any other element or integer or group of elements or integers.

Insulin - Dependent Diabetes Mellitus is a serious disease resulting from the destruction of insulin-secreting β - cells, probably mediated by T cells that recognise β-cell autoantigens. A major antigen implicated in T-cell mediated β-cell destruction characteristic of IDDM is glutamic acid decarboxylase (GAD), which occurs in two major isoforms, GAD 65 and GAD 67. These two isoforms have approximately 65% similarity at the amino acid sequence level. Subjects with IDDM or at high-risk of the disease show autoantibody and autoreactive T-cell responses to GAD insulin or both autoantigens. In NOD mice, an animal model for spontaneous IDDM, GAD is a dominant and early target antigen (Tisch *et al Nature 366*:72-75, 1993).

Identification of the immunodominant epitope(s) of pathogenic autoantigens involved in β-cell autoimmunity could lead to improved methods of diagnosis as well as therapeutic strategies to prevent IDDM.

In work leading up to the present invention, the inventors sought to identify immunodominant epitopes in GAD and proinsulin molecules in order to improve upon current diagnostic procedures and to further develop therapeutic and prophylactic compositions and treatment approaches for IDDM.

In accordance with the present invention, peptides were synthesised based on a thirteen amino acid region of high similarity between the sequences of human GAD 65 (amino acid residue numbers 506-518) and human proinsulin (amino acid residue numbers 24-36), which region of similarity also extends to human GAD 67 and mouse proinsulins and mouse GADs (Figure 1). The immunoreactivity of these peptides is identified in accordance with the present invention on the basis of interactivity of peripheral blood cells or T-cells obtained from the peripheral blood of subjects with pre-clinical or clinical IDDM, thereby forming the basis for a new range of diagnostic, therapeutic and prophylactic procedures for IDDM.

Accordingly, one aspect of the present invention provides a recombinant or synthetic peptide or chemical equivalents thereof of the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues; X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM). Preferred cells include but

are not limited to peripheral blood mononuclear cells (PBMCs), anticoagulated whole blood and tissue biopsy cells.

Reference to a "peptide" includes reference to a polypeptide or protein or parts thereof.

In a preferred embodiment X_2 comprises not less than about 10 and not greater than about 50, amino acid residues, more preferably not less than about 10 and not greater than about 30 amino acid residues and even more preferably not less than about 10 and not greater than about 15.

In a particularly preferred embodiment X_2 has either of the following amino acid sequences:

F F Y T P K T R R E A E D [SEQ ID NO:1]; or

F W Y I P P S L R T L E D [SEQ ID NO:2].

According to this preferred embodiment, there is provided a recombinant or synthetic peptide or chemical equivalent thereof comprising the sequence:

$$X_1X_2X_3$$

wherein

X_1 and X_2 may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues;

X_2 is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical IDDM and determining reactivity by an appropriate assay. Preferred cells include but are not limited PBMCs, anti-coagulated whole blood or tissue biopsy cells and determining reactivity by an appropriate assay.

The peptides of the present invention may be prepared by recombinant or chemically synthetic means. According to a preferred aspect of the present invention, there is provided a recombinant peptide which is preferentially immunologically reactive with

T-cells from individuals with clinical or pre-clinical IDDM, which is prepared by the expression of a host cell transformed with a cassette coding for the peptide sequences of the present invention. The peptide may be fused to another peptide, polypeptide or protein. Alternatively, the peptide may be prepared by chemical synthetic techniques, such as by the Merrifield solid-phase synthesis procedure. The synthetic or recombinant peptide may or may not retain GAD activity or proinsulin activity. Furthermore, although synthetic peptides of the formula given above represent a preferred embodiment, the present invention also extends to biologically pure preparations of the naturally occurring peptides or fragments thereof. By "biologically pure" is meant a preparation comprising at least about 60%, preferably at least about 70%, more preferably at least about 80% and still more preferably at least about 90% or greater as determined by weight, activity or other suitable means.

By "pre-clinical IDDM" as used herein means those subjects who may or may not be first degree relatives of someone with IDDM who have genetic and/or immune markers of pancreatic islet (β) cell autoimmunity. By "immune markers" is meant amongst other parameters known to those in the art to include circulating antibodies and/or T-cells reactive with islet (β) cell autoantigens.

By "derivatives" as used herein is taken to include any single or multiple amino acid substitution, deletion and/or addition relative to the naturally occurring amino acid sequence in the native molecule from which the peptide is derived including any single or multiple substitution, deletion and/or addition of other molecules associated with the peptide, including carbohydrate, lipid and/or other proteinacious moieties. Such derivatives, therefore, include glycosylated or non-glycosylated forms or molecules with altered glycosylation patterns.

By the term "reacting with T cells and modifying T-cell function" as used herein is taken to include T-cell activation, T-cell inactivation and/or T-cell death.

The present invention also covers chemical analogues of the subject peptides which include, but is not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives, during peptide synthesis and the use of cross-linkers and other methods which impose conformational constraints on the peptides or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5′-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation <u>via</u> O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The invention also extends to use of the peptides, or derivatives thereof of the present invention in the treatment of patients. In this latter aspect, such methods of treatment include their use as an adsorbent to remove autoantibodies or autoreactive cells from a patient, their use in direct administration to a patient as a means of desensitising or inducing immunological tolerance or other mechanisms to eliminate or diminish

reactivity of autoreactive T-cells or autoantibodies to IDDM autoantigens or to generate T-cell lines or clones to be used for or as therapeutic agents.

According to this aspect of the present invention, there is provided a method of treatment comprising administering to a subject an effective amount of a peptide or chemical equivalent thereof for a time and under conditions sufficient to remove or substantially reduce the presence or function in said subject of autoreactive T-cells and/or autoantibodies to IDDM autoantigens wherein the peptide comprises the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having clinical or pre-clinical Insulin-Dependent Diabetes Mellitus (IDDM). Preferred cells include but are not limited to peripheral blood mononuclear cells (PBMCs), anticoagulated whole blood and tissue biopsy cells.

The method of treatment contemplated herein includes, but is not limited to, the following examples. A first example of treatment is desensitisation or tolerance induction using an effective amount of synthetic peptide or derivative thereof to alter T-cell recognition of or response to GAD and/or pro-insulin and/or other IDDM antigens and/or induce T-cell suppression or regulation. This may be achieved by using the known effect of certain ultraviolet wavelengths, especially UV-B, to modify antigen presentation through the skin or transmucosal or systemic administration. Effective amounts of the peptides or derivatives thereof would be applied epicutaneously to the skin of subjects exhibiting peripheral blood T-cell reactivity to GAD or proinsulin peptides or polypeptides. After exposure of skin to UV-B

radiation, treatment would be repeated until such time that T-cell reactivity to GAD or proinsulin was suppressed.

A second example of treatment is to induce mucosal-mediated tolerance using an effective amount of the subject peptides or derivatives thereof to alter T-cell recognition of or response to GAD and/or pro-insulin and/or other IDDM antigens and/or induce T-cell suppression using an effective amount of peptide or derivative thereof to alter T-cell recognition of or response to GAD and/or pro-insulin and/or other IDDM antigens and/or induce T-cell suppression by the administration of the peptide or derivatives thereof by oral, aerosol or intranasal means amongst other routes of mucosal administration.

Another treatment involves application of the subject peptides to the skin together with one or more cytokines such as but not limited to TNFα or β. A further treatment involves systemic administration of soluble peptide via subcutaneous or intravenous routes to induce immunological tolerance. Yet another treatment involves T-cell immunisation whereby T-cell lines are generated to GAD or proinsulin peptide or polypeptide or fragments thereof by standard procedures, cells attenuated by fixation with agents such as glutaraldehyde or paraformaldehyde, washed under sterile conditions and re-injected into patients for a time and under conditions to cause suppression of the endogenous T-cell response to autoantigens. These approaches are applicable to the prevention of IDDM progression in asymptomatic subjects with pre-clinical IDDM or subjects with recent - onset clinical IDDM, as well as to the recurrence of IDDM in subjects who have received pancreas, islet cell or insulin-producing cell transplants. These approaches are also applicable to Stiff Man Syndrome (SMS) and other diseases where GAD and/or proinsulin is an autoantigen.

In accordance with the present invention, the effective amount of peptide is 0.1 μg to 10 mg per dose and preferably 1.0 μg to 1 mg per dose. A dose may comprise a single administration or protocol comprising single or multiple administration hourly, daily, weekly or monthly or at other suitable times. Administration may be by any

convenient means such as, but not limited to, intravenous, subcutaneous, epicutaneous, infusion, oral, topical, intranansal, aerosol suppository or intraperitoneal administration. The peptide may be administered alone or in combination with one or more other active molecules such as molecules which facilitate the activity or action of the peptide for example lipopolysaccharide (LPS), choleratoxin β-chain, Lymphocyte Functional Associated Antigen-3 (LFA-3), other adjuvants and in particular, tumour necrosis factor α (TNF-*α*), tumour necrosis factor β (TNF-*β*) or leukaemia inhibitory factor (LIF).

In yet a further embodiment, the present invention contemplates the use of the peptides described herein to measure reactivity of a subject's cells to the IDDM autoantigen. The peptides or derivatives thereof may be added in solution or bound to a solid support together with cells derived from peripheral blood or from tissue biopsies either unfractionated, fractionated or derived as continuous cell lines. Reactivity to the autoantigen may then be measured by standard proliferation assays such as incorporation of tritiated thymidine, standard cytotoxic assays such as release of marker radioactivity from target cells, measurements of expressed or secreted molecules such as surface markers, cytokines or other standard assays of cellular reactivity which are well known in the art.

According to this aspect of the present invention, there is provided a method of assaying the reactivity of a subject to IDDM autoantigen, said method comprising contacting a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having pre-clinical

or clinical Insulin-Dependent Diabetes Mellitus (IDDM) and determining reactivity by appropriate assay. In accordance with this assay, any cell type may be used but is preferably selected from PBMC's, anti-coagulated whole blood cells or tissue biopsy cells.

Preferably, the present invention contemplates a method of assaying the reactivity of a subject to IDDM autoantigen said method comprising contacting a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_2 may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues;

X_2 is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical IDDM and determining reactivity by an appropriate assay. Preferably, cells include but are not limited to peripheral blood mononuclear cells (PBMCs), anticoagulated whole blood and tissue biopsy cells.

In another embodiment of the present invention, there is provided a diagnostic kit for assaying T cells. Standard 96 - well plates, as used in ELISA, are pre-coated with a monoclonal antibody (MAb) to a T-cell cytokine such as γ-interferon (γ-IFN) with or without antigen. Alternatively, antigen is added in soluble form together with aliquots of peripheral blood, peripheral blood mononuclear cells or T-cells. Incubation is allowed to proceed for one or more days, the supernatant (comprising medium and plasma) and the cells are washed off, wells washed again and plates developed with a labelled second MAb to the cytokine such as anti-γ-IFN conjugated with alkaline phosphatase or horseradish peroxidase. Colorimetric reaction and read-out systems can then be utilised. Alternatively, soluble cytokines (eg: γ-IFN) are measured in the supernatant by standard assays such as ELISA; further it is possible to visualise microscopically by the ELISPOT technique individual spots on bottoms of wells representing cytokine produced at the single cell level thereby enabling the

frequency of peptide- epitope-reactive T-cells to be determined.

The present invention will now be further described with reference to the following non-limiting Figures and Examples.

In the Figures:

Figure 1 shows a comparison of the regions of similarity among mouse and human proinsulins and GADs. Similarities are boxed; identities within boxes are shaded. The C-terminus of the mature insulin B-chain and the pro-insulin cleavage site are indicated by the vertical line and arrow respectively.

Figure 2 is a graphical representation showing the level of cellular proliferation expressed as the delta score following the stimulation of peripheral blood mononuclear cells taken from IDDM at-risk (as described in Example 1) or control subjects with the following peptides: human GAD65 (residues 506-518); human proinsulin (residues 24-36); irrelevant control peptide; or tetanus toxoid (CSL Ltd., Melbourne, Australia).

Figure 3 is a graphical representation showing proliferation (mean + sem) of pbmc to proinsulin (aa 24-36) and insulin (aa 1-15) in pre-clinical and control subjects.

Figure 4 is a graphical representation showing IFN-gamma response (mean + sem) to proinsulin (aa 24-36) and insulin beta chain (aa 1-15) in pre-clinical and control subjects.

Figure 5 is a graphical representation showing IL10 response (mean + sem) to proinsulin (aa 24-36) and insulin beta-chain (aa 1-15) in pre-clinical and control subjects.

The following single and three letter abbreviations are used for amino acid residues:

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

EXAMPLE 1

Subjects

Subjects at-risk for IDDM were from the Melbourne Prediabetes Family Study, Victoria, Australia. Each was entered on the basis of having at least one first degree relative with IDDM and islet cell antibodies (ICA)≥20 JDF units and/or insulin autoantibodies (IAA)≥100nU/ml. All had normal fasting blood glucose and glycated hemoglobin and had had repeat antibody and metabolic tests at six monthly intervals.

Control subjects were HLA-DR matched, asymptomatic, and without history of IDDM.

All subjects gave informed, signed consent and the study was approved by the Ethics Committees of the Royal Melbourne Hospital and the Walter and Eliza Hall Institute of Medical Research. Details of Subjects are described in Table 1.

EXAMPLE 2

HLA typing and assays of ICA, IAA, GAD Ab, FPIR:

HLA Typing:

HLA class I (A, B, C) and HLA class II (DR,DQ) typing was performed using populations of T and B lymphocytes respectively. The cells were isolated from anticoagulated blood using magnetic beads (Dynal) coated with monoclonal antibodies to CD8 (class I) or a monomorphic determinant on the class II beta chain (class II). The enriched cell populations were typed in a standard microlymphocytotoxicity assay using a battery of 240 allosera for class I and 120 allosera for class II.

Antibody assays:

ICA were assayed using indirect immunofluorescence on blood group O donor pancreas. Titres, in JDF units, were determined by doubling dilution of positive sera

and comparison with standard sera run in each assay. The assay has been included in all International Diabetes Workshops and proficiency programs.

IAA were assayed by a radiobinding assay which has been internationally standardised. The upper limit for normal control sera is 40 nU insulin bound/ml serum.

GAD antibodies were assayed by immunoprecipitation of GAD enzymatic activity from piglet brain extract . The mean plus (three) 3 SD of 72 healthy subjects, 460nU/ml, was used to define the normal range.

First phase insulin release (FPIR):

FPIR was calculated as the sum of serum insulin concentrations at 1 and 3 minutes following the completion of intravenous glucose (0.5g/kg body weight) injected over 3 minutes.

EXAMPLE 3

T-cell proliferation assay

Blood was drawn from paired IDDM at-risk and HLA-DR matched controls at the same time (within 30 minutes) and processed similarly to reduce the effects of diurnal variation and handling artefacts. Peripheral blood mononuclear cells were isolated from heparinised whole blood by Ficoll-Paque (Pharmacia Biotech) density centrifugation, washed and resuspended in RPMI 1640 medium (Biosciences Pty Ltd) containing 20mM Hepes (CSL Ltd), 10^{-5} M 2-mercaptoethanol (BDH), penicillin (100U/ml), streptomycin (100μg/ml) and 10% v/v autologous plasma. Aliquots of 200μl ($2x10^5$ cells) were transferred into wells of a 96-well, round-bottomed plate (Falcon) and incubated in replicates of six with the following peptides at final concentrations of 10, 2, and 0.4μg/ml: human GAD65 (506-518), human proinsulin (24-36) (synthesised using an Applied Biosystems Model 431A synthesiser (ABI, Foster city, CA), and an irrelevant control peptide (CRFDPQFALTNIAVRK) (Macromolecular Resources, Fort Collins, CO). Tetanus toxoid (CSL Ltd,

Melbourne, Australia) at final concentrations of 1.8, 0.18 and 0.018 LfU/ml was used as a positive control. Twelve "autologous only" wells containing cells but without antigen were included as the background control. Plates were incubated at 37°C in a 5% v/v CO_2 humidified incubator for 6 days; 0.25μCi of [^{3}H]thymidine (ICN) was added to each well for the last 6 hours. The cells were than harvested onto glass fibre filters and incorporated radioactivity measured by beta-particle counting (Packard Model 2000 Liquid Scintillation Counter). The level of cellular proliferation was expressed as the delta score (DS=mean counts per minute (cpm) incorporated in the presence of antigen, minus the mean cpm of the "autologous only" wells).

EXAMPLE 4

T-cell Proliferative Responses

T-cell proliferative responses to the similar 13-mer peptides from proinsulin and GAD were compared for ten pairs of HLA-DR matched at-risk and control subjects. HLA-DR matching was thought to be important not only because of the specificity of peptide binding to MHC class II alleles but also because of the known association between MHC class II and IDDM. Therefore, T-cell responses would reflect IDDM rather than MHC specificity . Responses to the highest concentration of either peptide were significantly (proinsulin, $p<0.008$; GAD, $p<0.018$ - Wilcoxon one-tailed paired analysis) greater among IDDM at-risk than control subjects. The results are summarised in Table 2.

Reactivity to the proinsulin sequence was confined almost entirely to IDDM at-risk subjects, whereas some controls also responded to the GAD peptide (Table 2, Fig. 2). Both groups responded similarly to tetanus, and no subject reacted to the unrelated control peptide.

For six of these pairs (#1, 2, 3, 5, 6, 7) the assay was performed on a separate occasion, but using twice as many cells ($4x10^5$ per well). Exhaustion of the media resulted in unreliable results in three cases. In two of the other three (#5 and 6), the

results were consistent with those tabulated here, while in the third (#3) the at-risk subject displayed greater reactivity to both antigens at the higher cell number.

EXAMPLE 5

T-cell cytokine secretion assays

In a second cohort of 18 paired IDDM at-risk and HLA-DR-matched controls, PBMCs indicated as per Example 3 were incubated with human proinsulin 24-36 and human insulin B chain 1-15 each at 0.5, 5 and 50 μg/ml under the conditions as per Example 3. In addition to harvesting cells for the measurement of proliferation by [^{3}H] thymidine uptake after 6 days, as per Example 3, incubation media above the cells was sampled after 2 days for the measurement of IFN-γ and interleukin-(IL-) 10 by standard ELISA methods.

EXAMPLE 6

T-Cell Responses

T-cell proliferative and IFN-γ and IL-10 secretory responses to human proinsulin 24-36 and human insulin B 1-15 were compared for 18 pairs of HLA-DR matched IDDM at-risk and control subjects. As per Example 4, there was a significantly greater ($p=0.003$) proliferative response of IDDM at-risk subjects to the proinsulin peptide (Figure 3). In addition, both IFN-γ and IL-10 secretion in response to the proinsulin peptide were significantly increased ($p=0.005$ and $p=0.001$, respectively) compared to matched control subjects (Figures 4, 5).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Table 1

Subject #	Age	Years Follow-up	HLA				ICA *
			A	B	DR	DQ	
1	14	1.6	1	8	3	2	160,69,56
2	23	4.8	2	44,55	3,4	5,8	55,37,14,6,5,5
3	22	6.8	2,28	7,8	3,4	2,8	37,37,37,37,52,30,58,46,26
4	13	1.3	1,11	8,27	3,4	2,8	160,190
5	25	5.5	2	44,62	4,11	7,8	0,19,18,16,22,0,0
6	20	5.5	1,2	8,62	3,4	2,8	19.19.104.86.8
7	18	1.7	1,3	8,18	3	2	69,69
8	9	3.2	1,2	8,44	3,4	2,8	160,160,160,160
9	10	2.8	1,2	8,27	3,4	2,8	160,160,120,24
10	14	4.8	1,32	8,14	4,7	2,8	14,13,51,18

(Continued...)

Table 1 (...continued)

Subject #	Age	Years Follow-up	IAA †	GAD Ab £	FPIR ¶
1	14	1.6	4,30,-20		118,155
2	23	4.8	-25,9,41,-2,0,44	278,602	124,113,57
3	22	6.8	8,9,2,31,7,9,-41,-1,64	1637,2259,634,1535	183,155,140,161,56
4	13	1.3	84.280		79,91
5	25	5.5	45,31,42,60,29,130,30	736,936,1336,790,810	137,143,68,15
6	20	5.5	480,560,400,130,300	937,2258,2389	105,238,165,128
7	18	1.7	13,20		44,47
8	9	3.2	-2,-26,36,59	2300,1830	118,129,87
9	10	2.8	2,29,14,120	1525,1388	26,56,29
10	14	4.8	240,490,470,1000	432	318,181,165

* ICA=islet cell antibody titres (JDF units)

† IAA=insulin autoantibody titres (nU insulin bound/ml serum)

£ GADAb=glutamic acid decarboxylase autoantibody titres (nU/ml)

¶ FPIR=first phase insulin release (sum of serum insulin concentrations at 1 and 3 minutes following completion of glucose injection)

Table 2

Pair #		Delta Scores*							
		Autologous		Proinsulin 10 µg/ml		Proinsulin 2 µg/ml		Proinsulin 0.4 µg/ml	
		At Risk	*Control*	*At Risk*	*Control*	*At Risk*	*Control*	*At Risk*	*Control*
1		881	2979	1391	0	459	0	1040	0
2		236	389	351	0	0	0	33	0
3		6515	217	0	64	355	43	0	0
4		595	1347	104	0	0	0	288	0
5		1745	1269	694	0	0	0	0	0
6		1007	265	397	98	65	380	0	0
7		1392	454	467	93	0	0	0	0
8		9993	308	2128	0	1367	0	0	0
9		598	135	0	0	265	13	0	0
10		597	870	56	21	0	22	0	0
	Mean	2355.8	823.4	558.7	27.6	251.1	45.8	136.0	0
	Std. Error	1025.7	276.2	219.5	13.0	135.3	37.4	104.4	0
	Wilcoxon P-Value (One-Tailed)			0.008		0.125		0.054	

(Continued...)

Table 2 (...continued)

Pair #		Delta Scores*					
		GAD 10 µg/ml		GAD 2 µg/ml		GAD 0.4 µg/ml	
		At Risk	*Control*	*At Risk*	*Control*	*At Risk*	*Control*
1		579	0	516	0	768	0
2		3263	0	190	0	199	0
3		0	77	0	5	0	25
4		0	0	10	0	0	0
5		1275	20	394	120	53	30
6		1679	992	220	216	77	195
7		2313	1365	0	0	0	70
8		0	0	0	0	0	0
9		1251	337	0	0	255	21
10		65	391	0	1441	0	0
	Mean	1042.4	318.1	133.0	178.2	135.3	34.1
	Std. Error	357.1	153.0	60.5	142.2	76.0	19.2
	Wilcoxon P-Value (One-Tailed)	0.018		0.199		0.199	

* Delta Score=mean of six replicate wells minus mean of twelve autologous wells (if less than 0, shown as 0)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (Other than US): AMRAD OPERATIONS PTY LTD
(US only): HARRISON, L; HONEYMAN, M; RUDY, G; and LEW, A.

(ii) TITLE OF INVENTION: Immunoreactive and Immunotherapeutic Molecules"

(iii) NUMBER OF SEQUENCES: 2

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 20-FEB-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PN1239/95
(B) FILING DATE: 20-FEB-1995
(A) APPLICATION NUMBER: PN5172/95
(B) FILING DATE: 04-SEP-1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Phe Phe Tyr Thr Pro Lys Thr Arg Arg Glu Ala Glu Asp
5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Phe Trp Tyr Ile Pro Pro Ser Leu Arg Thr Leu Glu Asp
5 10

CLAIMS:

1. A recombinant or synthetic peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:
X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;
X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM).

2. A peptide molecule according to claim 1 wherein X_2 comprises from 10 to 50 amino acid residues.

3. A peptide molecule according to claim 2 wherein X_2 comprises from 10 to 30 amino acid residues.

4. A peptide molecule according to claim 3 wherein X_2 comprises from 10 to 15 amino acid residues.

5. A peptide molecule according to claim 1 or 2 or 3 or 4 wherein X_2 comprises the amino acid sequence: FFYTPKTRREAED.

6. A peptide molecule according to claim 1 or 2 or 3 or 4 wherein X_2 comprises the amino acid sequence: FWYIPPSLRTLED.

7. A recombinant or synthetic peptide or chemical equivalent thereof comprising the sequence:

$$X_1X_2X_3$$

wherein:

X_1 and X_2 may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues;

X_2 is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having pre-clinical or clinical IDDM.

8. A method of assaying the reactivity of a subject to IDDM autoantigen said method comprising contacting a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM) with cells from said subject and determining reactivity by an appropriate assay.

9. A method according to claim 8 wherein the cells are selected from the group comprising PBMCs, anti-coagulated whole blood and/or tissue biopsy cells.

10. A method according to claim 8 or 9 wherein an appropriate assay includes proliferation assay, cytotoxic assays, celular reactivity or combination thereof.

11. A method according to claim 8 wherein X_2 comprises from 10 to 50 amino acid residues.

12. A method according to claim 11 wherein X_2 comprises from 10 to 30 amino acid residues.

13. A method according to claim 12 wherein X_2 comprises from 10 to 15 amino acid residues.

14. A method according to claim 8 or 9 or 10 or 11 or 12 wherein X_2 comprises the amino acid sequence: FFYTPKTRREAED.

15. A method according to claim 8 or 9 or 10 or 11 or 12 wherein X_2 comprises the amino acid sequence: FWYIPPSLRTLED.

16. A method of assaying the reactivity of a subject to IDDM autoantigen said method comprising contacting a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_2 may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues;

X_2 is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical IDDM with cells from said subject and determining reactivity by an appropriate assay.

17. A method according to claim 16 wherein the cells are selected from the group comprising PBMCs, anti-coagulated whole blood and/or tissue biopsy cells.

18. A method according to claim 16 or 17 wherein an appropriate assay includes proliferation assay, cytotoxic assays, celular reactivity or combination thereof.

19. Use of a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM) to assay reactivity of a subject to IDDM autoantigen by contacting said peptide or its chemical equivalent to cells from said subject and determining reactivity by an appropriate assay.

20. Use according to claim 19 wherein the cells are selected from the group comprising PBMCs, anti-coagulated whole blood and/or tissue biopsy cells.

21. Use according to claim 19 or 20 wherein an appropriate assay includes proliferation assay, cytotoxic assays, celular reactivity or combination thereof.

22. Use according to claim 19 wherein X_2 comprises from 10 to 50 amino acid residues.

23. Use according to claim 22 wherein X_2 comprises from 10 to 30 amino acid residues.

24. Use according to claim 23 wherein X_2 comprises from 10 to 15 amino acid residues.

25. Use according to claim 19 or 20 or 21 or 22 or 23 or 24 wherein X_2 comprises the amino acid sequence: FFYTPKTRREAED.

26. Use according to claim 19 or 20 or 21 or 22 or 23 or 24 wherein X_2 comprises the amino acid sequence: FWYIPPSLRTLED.

27. Use of a peptide or chemical equivalent thereof comprising the formula:

$$X_1X_2X_3$$

wherein:
X_1 and X_2 may be the same or different and each is an amino acid sequence comprising from 0 to 15 naturally or non-naturally occurring amino acid residues;
X_2 is selected from FFYTPKTRREAED and FWYIPPSLRTLED or a derivative or chemical equivalent thereof and wherein said peptide is capable of reacting with T cells and modifying T-cell function when incubated with cells from subjects with pre-clinical or clinical IDDM to assay reactivity of a subject to IDDM autoantigen by contacting said peptide or its chemical equivalent with cells from said subject and determining reactivity by a proliferation assay.

28. Use of a peptide or chemical equivalent according to claim 27 wherein the cells are selected from the group comprising PBMCs, anti-coagulated whole blood and/or tissue biopsy cells.

29. Use of a peptide or chemical equivalent according to claim 27 or 28 wherein an appropriate assay includes proliferation assay, cytotoxic assays, celular reactivity or combination thereof.

30. A method of treatment comprising administering to a subject an effective amount of a peptide or chemical equivalent thereof for a time and under conditions sufficient to remove or substantially reduce the presence in said subject of autoreactive T-cells and/or autoantibodies to IDDM autoantigens wherein the peptide comprises the formula:

$$X_1X_2X_3$$

wherein:

X_1 and X_3 may be the same or different and each is an amino acid sequence comprising from 0 to 40 naturally or non-naturally occurring amino acid residues;

X_2 is any amino acid sequence of from 10 to 100 residues derived from, homologous to or contiguous within amino acids 506 to 518 inclusive or derivatives thereof of human GAD65 and/or amino acids 24 to 36 inclusive or derivatives thereof of human proinsulin; and wherein said peptide molecule is capable of reacting or modifying T-cell function when incubated with cells from subjects having pre-clinical or clinical Insulin-Dependent Diabetes Mellitus (IDDM).

31. A method according to claim 30 wherein X_2 comprises from 10 to 50 amino acid residues.

32. A method according to claim 31 wherein X_2 comprises from 10 to 30 amino acid residues.

33. A method according to claim 32 wherein X_2 comprises from 10 to 15 amino acid residues.

34. A method according to claim 30 or 31 or 32 or 33 wherein X_2 comprises the amino acid sequence: FFYTPKTRREAED.

35. A method according to claim 30 or 31 or 32 or 33 wherein X_2 comprises the amino acid sequence: FWYIPPSLRTLED.

36. A pharmaceutical composition comprising a recombinant peptide or equivalent thereof according to claim 1 or 7 and one or more pharmaceutically acceptable carriers and/or diluents.

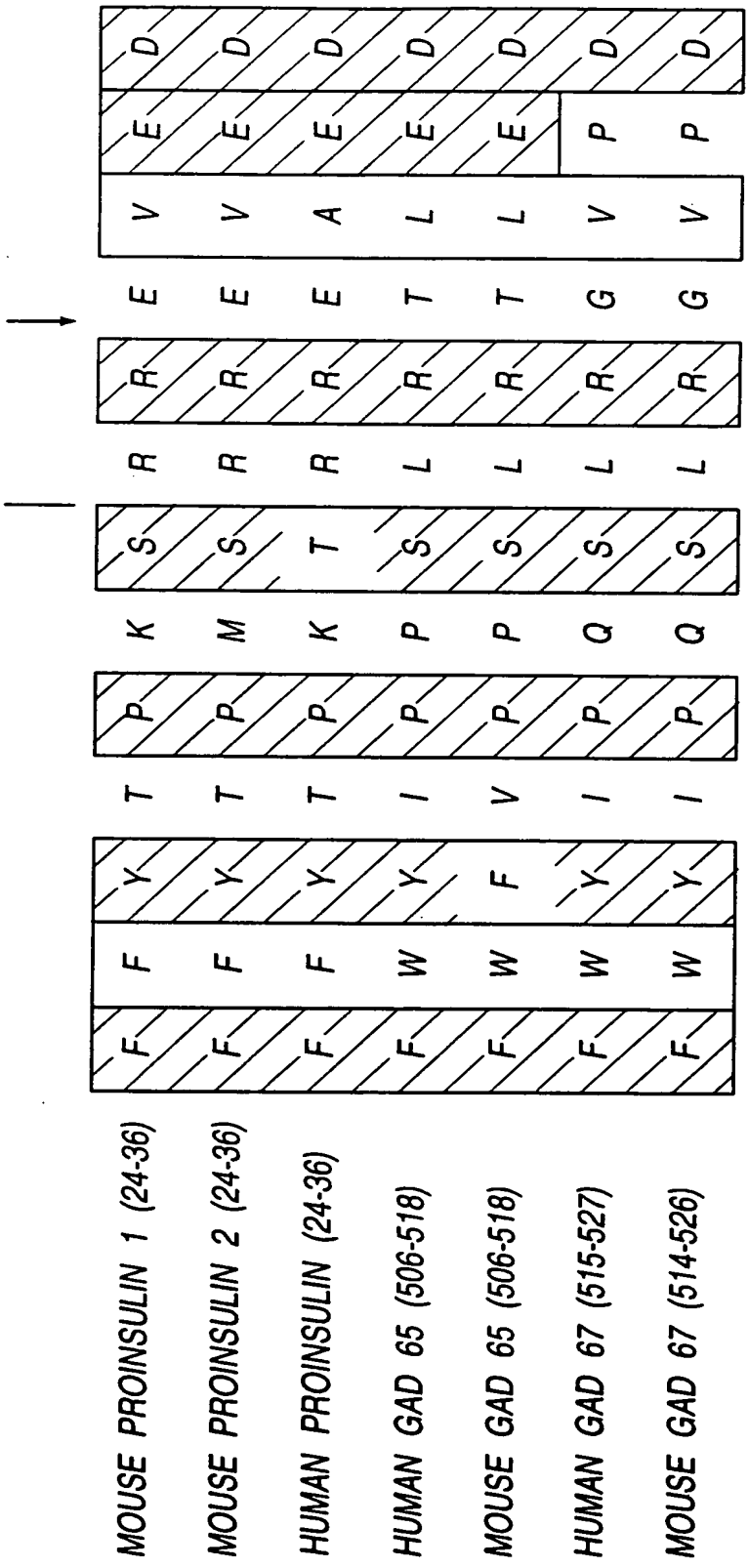
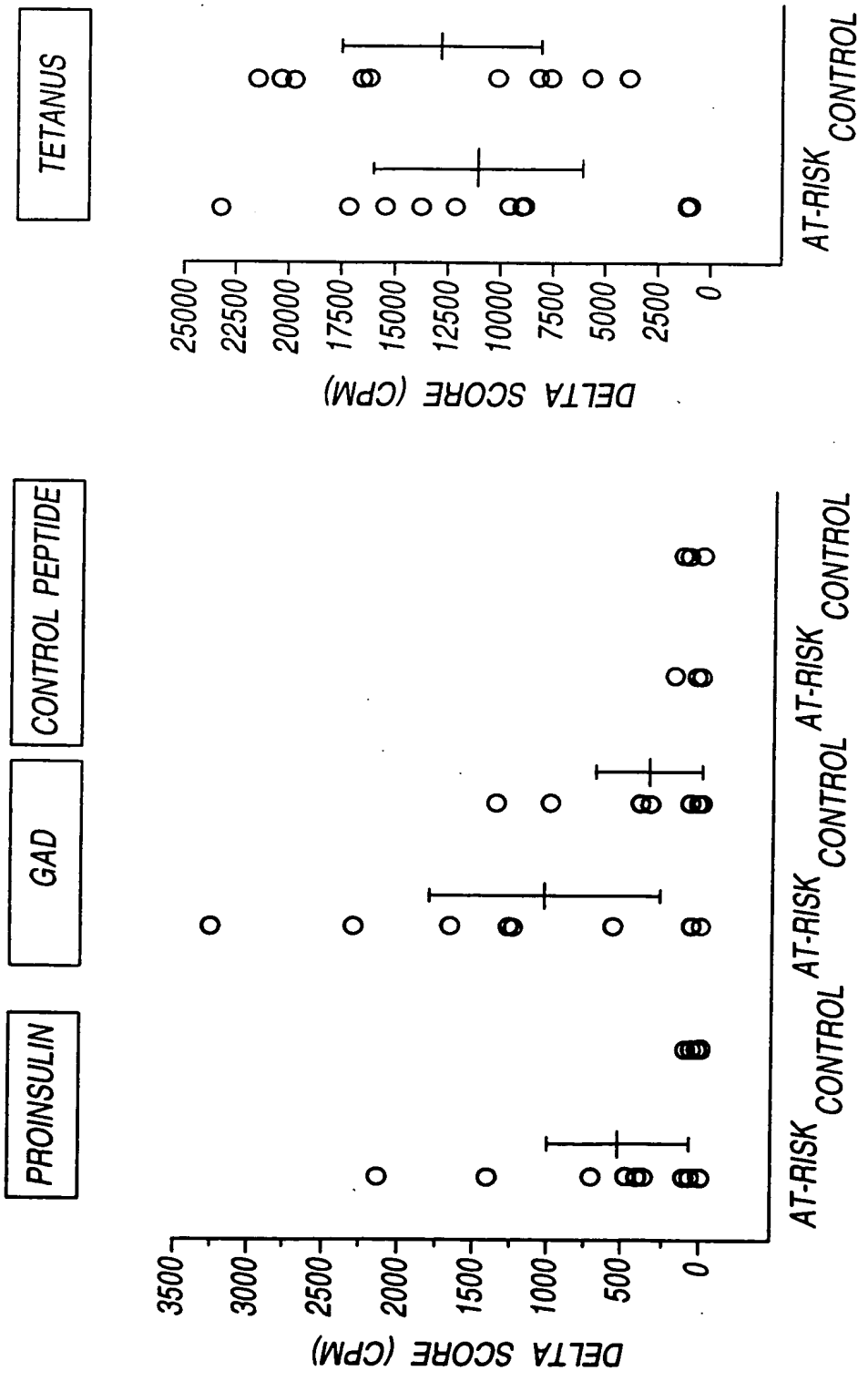

MOUSE PROINSULIN 1 (24-36)
MOUSE PROINSULIN 2 (24-36)
HUMAN PROINSULIN (24-36)
HUMAN GAD 65 (506-518)
MOUSE GAD 65 (506-518)
HUMAN GAD 67 (515-527)
MOUSE GAD 67 (514-526)
F F Y T P K S R E V E D
F F Y T P M S R E V E D
F F Y T P K T R E A E D
F W Y I P P S L T L E D
F W F V P P S L T L E D
F W Y I P Q S L G V P D
F W Y I P Q S L G V P D

Fig. 1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GAD
CONTROL PEPTIDE
TETANUS
DELTA SCORE (CPM)
3500
3000
2500
2000
1500
1000
500
0
AT-RISK
CONTROL
AT-RISK
CONTROL
AT-RISK
CONTROL
DELTA SCORE (CPM)
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22500
20000
17500
15000
12500
10000
7500
5000
2500
0
AT-RISK
CONTROL

Fig. 2

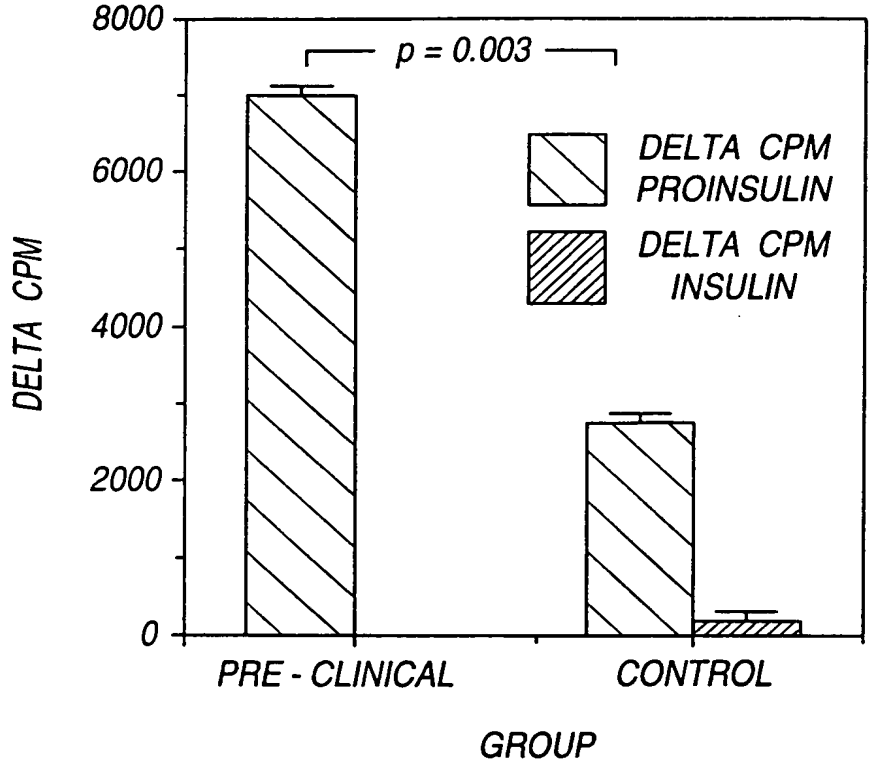
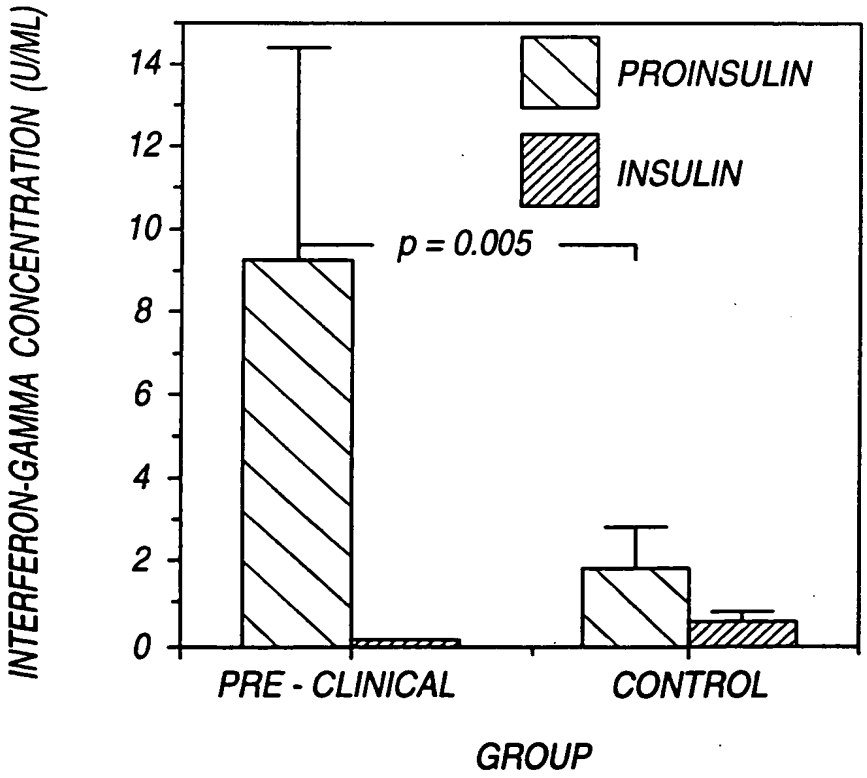

8000
6000
4000
2000
0
DELTA CPM
p = 0.003
DELTA CPM PROINSULIN
DELTA CPM INSULIN
PRE - CLINICAL
CONTROL
GROUP

Fig . 3


14
12
10
8
6
4
2
0
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PROINSULIN
INSULIN
p = 0.005
PRE - CLINICAL
CONTROL
GROUP

Fig . 4

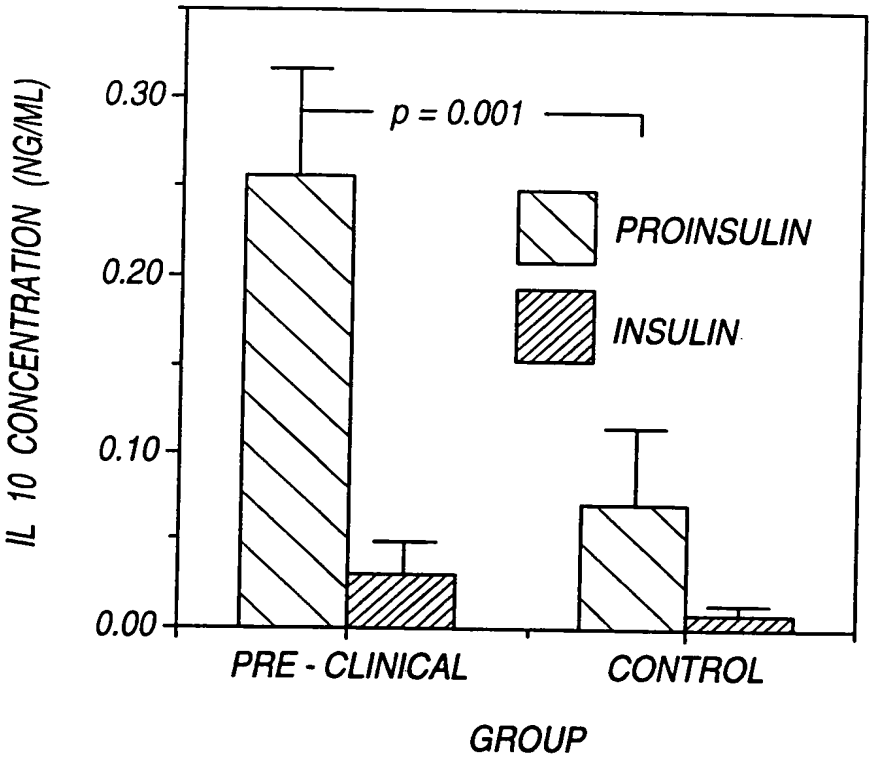
IL 10 CONCENTRATION (NG/ML)
0.30
0.20
0.10
0.00
p = 0.001
PROINSULIN
INSULIN
PRE - CLINICAL
CONTROL
GROUP

Fig . 5

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00085

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/62, 14/725, 14/47, 7/06, 7/08; G01N 33/68, 33/564; C12Q 1/25; A61K 38/43, 38/10, 38/28 //C12Q 1/02; C12R 1:91

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6]: C07K, A61K, C12Q, G01N. All through electronic databases. Chemical Abstracts

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Medline Through electronic database

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, Medline Keywords: SS1: GAD or GLUTAMIC (W) ACID (W) DECARBOXYLASE# SS2: PROINSULIN# or PRO(W)INSULIN# SS3: IDDM or DIABETES(W)MELLITUS SS4: 1 or 2 SS5: 3 and 4 STN SEARCH: FYTPKTRRE; YTPKTRREAE; TPKTRREAED; FWYIPPSLRT; WYIPPSLRTL; YIPPSLRTLE; IPPSLRTLED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 95/07992 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 25 March 1995, IPC[6] C12N, C07K, G01N, A61K See entire document especially page 26 line 7 - page 28 line 9, Examples, Table 11, claims, Figure 3	1-3,6-12,15-23, 26-32,35,36
X,Y	WO 92/05446 (REGENTS OF THE UNIVERSITY OF CALIFORNIA) 2 April 1992, IPC[5] G01N, C12N, C07H, A61K See entire document especially Example 3 and Figures 2, 3 and 4	1,5-10,14-17 19,20,25-28, 30,34-36

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 1996	1ST MAY 1996.
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer **ROBYN PORTER** Telephone No.: (06) 283 2318

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/14485 (AMRAD CORPORATION LIMITED) 3 September 1992, IPC[5] A61K, C07K, C12N, G01N See entire document	1,6-8,15,16,18, 26,27,30,35,36
X	WO 94/12529 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 9 June 1994, IPC[5] C07K, G01N See entire document, especially page 35 line 18 to page 38 line 6, Example 7, claims 21, 37, 42, 45, 47 and 50 and Figure 1	1,8-10, 19-21,30,36
X	K. Daw & A.C. Powers: "Two Distinct Glutamic Acid Decarboxylase Auto-Antibody Specificities in IDDM Target Different Epitopes", Diabetes, vol 44, no 2, 216-220, February 1995 See entire document including Figure 3	8,14-16,19, 25-27
Y	L. Mauch et al: "Characterization of a linear epitope within the human pancreatic 64-kDa glutamic acid decarboxylase and its autoimmune recognition by sera from insulin-dependent diabetes mellitus patients". Eur. J. Biochem., 212, 2, 597-603 (1993)	1,6-8,15, 16,19,26,27
P,X	WO 95/07464 (UNIVERSITY OF WASHINGTON) 16 March 1995, IPC[6] G01N, C07K See abstract, Examples 3 and 4, Sequence ID Nos 1 and 2 and claims	8-10,19-21
X	WO 92/20811 (ZYMOGENETICS & THE BOARD OF REGENTS OF THE UNIVERSITY OF WASHINGTON) 26 November 1992, IPC[5] C12P, C12N, C12Q, C07K, C07H, A01N See page 15 line 27 - page 16 line 36, page 20 line 29 - page 21 line 29, Example 3, claims 16, 21 and 25 and Figure 2	16-21, 26-30,35,36

INTERNATIONAL SEA[redacted]EPORT

[redacted]nternational Application No.

Information on patent family members

PCT/AU 96/00085

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9507992	AU	79201/94				
WO	9205446	AU	88771/91	AU	21706/95	CA	2040555
		EP	502188	US	5475086	AU	15163/92
		CA	2070004	EP	519469	JP	7070182
WO	9214485	AU	12748/92	CA	2104225	EP	572478
WO	9412529	EP	701569				
WO	9507464	AU	76441/94				
WO	9220811	AU	19976/92	CA	2103159	EP	585356
		IL	101872	NZ	242747		

END OF ANNEX